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Supplemental Information

Antigen Identification for Orphan T Cell Receptors Expressed on Tumor-Infiltrating Lymphocytes

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Cluster 1 Peptides	Cluster 1 Predictions	Cluster 2 Peptides	Top 10 Cluster 2 Predictions
SMLGIGIVPV	EAAGIGILTV	MMWDRGMGLL	MLWDVQSGQM
SMAGIGIVDV	TLGGIGLVTV	IMEDVGWLNV	LLLQVGLSLL
NMGGLGIMPV	ILLGIGIYAL	MMWDRGLGMM	SLEDVVMLNV
NLSNLGILPV	ILSGIGVSQV	ILEDRGFNQV	MLEDRDLFVM
SMLGIGIYPV	IMGNLGLIAV	LMFDRGMSLL	MLEDMSLGIM
TMAGIGVHVV	MAGNLGIITL	LMLDFDGSLL	SLENRGLSML
SMAGIGTLVV	IMGNLGLIVL	IMEDRGSLNM	ILDDGGFLLM
SMSGLGILPM	ILAGLGTSLL	LMNDMGFHIV	LLWNFGLLIV
SMAGIGIVPV	ELGGLKISTL	IMEDRGSGEM	LLFDISFLML
SMLGIGIVDV		LMWDVGLSIM	IMGDRNRNLL
NMAGIGMGTV		SMWDRGTFIM	
SMLGIGILPV		LMLDRGSPNM	
SLSGIGISAV		IMFDRGIGIM	
DLAGLGLYPV		ILFDRGMNLM	
NMAGIGIIQV		MLLDRGLSLM	
NMGGLGILPV		IMEDRGSLIL	
SMAGIGIYPV		LMRDYQLLQV	
NLSNLGIVPV		LMFDRGMSVL	
IMLGIGIDTL		LMEDIGRELV	
NLSNLGIMPV		ILEDRGMGLL	
SMLGIGIVLV		MMDQFNGLMM	
SMAGIGVHVV		IMWDRDYGVM	
NMAGIGILTV		MMWDRGFNQV	
MMAGIGIVDV		IMSMSVSNYL	
NMGGLGIVPV		AMGDGSYLLM	
SMLGIKIVPV		SMWDRGMGLL	
ELSGLGIQTV		MMENRGSGAL	
SMLGIGILPM		LMWDSGLELM	
SMAGIGILPV		SMWDRGLGMM	
SMLGIGIVPV		LMWDVGWLNV	
		MMWDRGTFIM	
		MMWDRGIVPV	
		ILFDRGMNLM	

Table S1. DMF5 selection data and human target prediction.

The sequences identified from the round 3 deep-sequencing of the DMF5 10mer library selections after clustering by reverse hamming distance. Using these clusters, predictions were made on the Uniprot database using 2014PPM. The 9 predictions for the ‘GIG’ cluster and top 10 predictions for the ‘DRG’ cluster are listed. Related to Figure 2 and Figure S1.

NKI2 9mers	NKI2 10mers		NKI2 11mers
VMISHENFM	VMNGDSGTFL	TLMSRSDLFL	ILSNRGHEVFV
TMQSHEVML	YMAVRSENFM	ILNSRDEAMM	ILSNRGHENFM
TMQSHENFM	RMPNKQENFV	ALNSRDEAMM	ILSNRGHDVFM
VMQSHEVML	IMDSKSEHFM	ALDSRLEFFV	ILSNRGHEIFL
VMISHEIFL	IMDSREEVFV	VMDSRLEFFV	ILSNRGHEYFL
IMTSHEVML	IMDSRSEHFM	ALDSRSELFL	
IMTSHEVMM	GMDSRAEVFM	AMYSNSDFMV	
VMESHDVFM	ALDSRSEYFL	VMDSRLEHFM	
IMNSHEVMM	KMANRDENFV	SMNSRSEHFM	
SMNSHEVMM	RLDGQDTKFM	SMNSKSENFL	
KMNSHEVMM	LMDSRSEHFM	VLDSSSSSFL	
AMQGHEYFL	IMNSRSELFL	ALDSRSENFL	
AMQGHEIFL	MMNVRSELFV	ALDSKSENFL	
VLQSHEVSM	TMNVRSELFV	ALDSRSEIFL	
AMQSHEVTL	KMNSRSELFL	SMNSRADMFV	
LMSGDYQFV	TMNVRSEHFM	SMYSRQEMMV	
TMHNHEVMM	SMNSRSELFL	RMWSRSEDMV	
VMHNHEVMM	KMNSRSEHFM	VLRARSDVFV	
TMTGHEVFM	TMQSHDASFL	ALDSREEVFV	
TMTGHEVFV	VMQGHDASFL	SMNSREEIFL	
VMQGHESFL	KMNSHSGTFL	SMSGFSESFV	
VMISHEVML	KMNGKSEDFM		
TMTGHEVML	DMDNRLDRDM		
SMVGMEHSM	IMDSKSEIFL		
AMQGHEHFM	SMNSHSGTFL		
VMEGDYWFL	SMNSREEHFM		
SMQSHEWML	IMNSHSGTFL		
YMQTHESFM	IMDSKSENFL		
	AMDSKSENFL		
	IMDSRADMFV		
	SMNSREEVFV		
	KMNSREEVFV		
	ALDSRSEHFM		
	AMDSRSEHFM		
	AMDSRADMFV		
	LMDSRSQIFV		
	GMTSRSDYMV		
	VMNSRSEHFM		
	VMNSRSDWFL		
	YMNSHDPYTV		
	RMDSRSQDFV		
	RMEAHSSHFV		

Table S2. NKI2 selection data by peptide length.

The sequences identified from the round 3 deep-sequencing of the NKI2 library selections listed by peptide length. Related to Figure 3.

HLA	Patient A	Patient B
A	2:01	2:01
	2:01	2:06
B	7:02	15:01
	15:01	35:01:00
C	ND	ND
	ND	ND
DRB1	1:01	4:07
	4:04	4:07
DRB345	4*01:01	4*01:01
	ND	4*01:01
DQA	1:01	3:01
	3:01	3:01
DQB	3:02	3:02
	5:01	3:02

Table S3. Patient HLA typing results.

The HLA type of two patients with colorectal adenocarcinoma. ND = Not determined. Related to Figure 4.

Patient A					
Tumor	Healthy	Vβ	CDR3β	Vα	CDR3α
23	12	TRBV7-2	CASSLGLEQFF	TRAV8-3	CAGGGGADGLTF
6	0	TRBV7-3	CASSLGGGHTEAFF	TRAV19	CALSEAEAAGNKLTF
5	0	TRBV7-9	CASSLVNGLGYTF	TRAV19	CALSEAGMDSNYQLIW
4	0	TRBV15	CATSRDRGQDEKLFF	TRAV14/DV4	CAMREGRYSGAGSYQLTF
4	0	TRBV9	CASSADTGVNQPQHF	TRAV10	CVVTETNAGKSTF
4	0	TRBV10-1	CASSRDTVNTEAFF	TRAV19	CALSEARGGATNKLIF
1	0	TRBV20-1	CSARDYQGSQPQHF	TRAV12-2	CAVNSGNTGKLIF
1	0	TRBV20-1	CSARDYQGSQPQHF	TRAV20	CAVPFLYNQGGKLIF
1	0	TRBV9	CASSADTGVNQPQHF	TRAV12-2	CAVNDFNKFYF
Patient B					
Tumor	Healthy	Vβ	CDR3β	Vα	CDR3α
35	0	TRBV11-2	CASSQGVGQFKNTQYF	TRAV12-2	CAVETSNTGKLIF
23	0	TRBV7-2	CASSLSGRQGGSYEQYF	TRAV29/DV5	CAASSTGNQFYF
21	0	TRBV9	CASSSSGGLVDTQYF	TRAV19	CALSAGASGAGSYQLTF
20	0	TRBV2	CASMGRSYGYTF	TRAV39	CALMNYGGATNKLIF
16	0	TRBV11-3	CASSLETGTAIYEQYF	TRAV13-1	CAADNNNARLMF
12	0	TRBV11-3	CASSPSGLAGSNLGNEQFF	TRAV19	CALSSRGSTLGRLYF
11	0	TRBV5-1	CASSRIDSTDTQYF	TRAV4	CLVGEVGTASKLTF
10	0	TRBV19	CASSIPRGSSQPQHF	TRAV12-2	CAVDSGGYNKLIF
8	0	TRBV10-3	CAIKGGDRGVNTEAFF	TRAV14/DV4	CAMREPNNAGNMLTF
4	3	TRBV20-1	CSARLASYNEQFF	TRAV12-2	CAVRRATDSWGKLQF
1	1	TRBV10-1	CASSRDFVSNEQYF	TRAV19	CALSEARGGATNKLIF

Table S4. Orphan TCRs screened on the HLA-A*02:01 library.

The TCRs screened on the HLA-A*02:01 library. TCR sequences were chosen based on clonality in the tumor, phenotypic profile, exclusivity to the tumor, and additionally by related TCR sequences. The number beneath tumor and healthy labels indicate the number of times a paired TCR sequence was seen from this tissue. Related to Figures 5 and 6.

		TCR1A			
Name	Sequence	Library	2014PPM	2017PWM	2017DL
91	SMGVTYEM	1			
92	YMGVSYEM	2			
93	YMGVVYEM	3			
94	KMGVTYEM	4			
95	FMGVTYEM	6			
9DL1	NMEVTYEI			1	1
9DL2	FITVTEEI			82	2
9DL3	HIQVTNEI				3
9DL4	HLIVSYEL			27	4
9DL5	HLGVTKEL			5	5
9PWM1	RLGVTYFV			2	
9PWM2	YLPVTYHI			3	60
9PWM3	GLGQTYEI			4	19
Ex1	EYGVSYEW				
Ex2	EYGVQNYV				
9EX5	EMGVSYEM				

Table S5. Peptide prediction results for TCR 1A.

Peptides tested to stimulate TCR 1A cells. Peptides are derived from the library, 2014PPM, 2017PWM and 2017DL predictions and the rank of the peptide from the predictions or library selections are shown. Cells are left intentionally blank. Related to Figure 7, S3, and S4.

		TCR 2A				TCR 3B			
Name	Peptide	Library	2014PPM	2017PWM	2017DL	Library	2014PPM	2017PWM	2017DL
1s1	LMDMHNGQ	1							
1s2	RLDAMNGQ	2							
1s3	RMDYNNMQ	3							
1s4	SMDTFQGQ	4				9			
1s5	GMDYHNGH	5							
2s1	TMDFYQGQ	41				1			
2s2	KMDYFSGQ	42				2			
2s3	SMDWFQGQ					3			
2s4	LMDYWQGQ					4			
2s5	NMMWFQGQ					5			
1sj1	VLDLFQGQ		1	6				3	2
1sj2	MMDFFNAQ		2	1				2	160
1sj3	LLNLNNGQ		3	28					
1sj4	QMDYEEGQ		4	5				1	100
1sj5	GLSSQNGQ		5						
2sj1	TLHYYEMH		154				1		
DG1	VIDFLNNQ			21	10			44	
1sDL1	VIDQLNGQ			3	1			24	154
1sDL2	VVDFLKGQ			31	2			8	16
1sDL3	QVDGESVQ				3				
1sDL4	HIDYFNNQ			2	4			32	49
1sDL5	VVLGEQVQ				5				
DG2	HVDYKNVQ			9	9				
1sPWM4	AISMFNGQ			4				197	166
DG3	RIDPHDGQ			8				94	11
DG4	SLDSYNAQ		10	37					
2sDL1	TIHSYQGQ							8	1
2sDL3	SLDFYQGH							7	3
2sDL4	IIDAFKGQ								4
2sDL6	RIDDGQGQ							365	6
2sDL7	TILQYSGQ							70	7
2sPWM4	LVDFWEAQ			40				4	
2sPWM5	TIAHYSGQ							5	30
2sPWM6	KVDFPQDQ							6	97
DG6	TIRELQGQ							148	13
DG7	FIDFVQRQ							130	14

Table S6. Peptide prediction results for TCRs 2A and 3B.

Peptides tested to stimulate TCRs 2A and 3B cells. Peptides are derived from the library, 2014PPM, 2017PWM, and 2017DL predictions and the rank of the peptide from the predictions or library selections are shown. Cells are left intentionally blank. Related to Figure 7, S3, and S4.

		TCR4B			
Name	Peptide	Library	2014PPM	2017PWM	2017DL
B9_1	RMEQVDWTV	1			
B9_2	KLEFMDWRL	2			
B9_3	WLDNFELCL	3			
B9_4	TLEYMDWLV	4			
B9_5	EMMLFDWKV	5			
B9DL1	KLEQLDWTV			2	1
B9DL2	TMETIDWKV			1	2
B9DL3	DLEQMEQTV			59	3
B9DL4	TLEELDWCL			102	4
B9DL5	TLEDMAWRL			75	5
B9DL6	NVEEMDWLI			4	6
B9DL7	NVEEMDWMV			3	7
B9DL8	LLEDLDWDV				8
B9DL9	TLEAMNTTV			914	9
B9DL10	VLEEVDWLI			122	10
B9DL11	WLEDVEWQV			18	11
B9PWM1	KMENFDKTV			5	50
B9PWM2	NMEYMTWDV			6	36
B9PWM3	FVENVEWRV			7	399
B9PWM4	NVEYYDIKL			8	
B9PWM5	HLEQVDKAV			9	34
B9PWM6	EMEQVDAVV			10	287

Table S7. Peptide prediction results for TCR 4B.

Peptides tested to stimulate TCR 4B cells. Peptides are derived from the library, 2014PPM, 2017PWM, and 2017DL predictions and the rank of the peptide from the predictions or library selections are shown. Cells are left intentionally blank. Related to Figure 7, S3, and S4.

Name	Primer Sequence
ILL_F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGA
ILL_R	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTC
ILL_B2M_R	ATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCAGGATGTCTACTGTAAACTTG
ILL_BC_1	CTACACGACGCTCTTCCGATCTNNNNNNNNTTAGCATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_2	CTACACGACGCTCTTCCGATCTNNNNNNNNTTATGCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_3	CTACACGACGCTCTTCCGATCTNNNNNNNNTTCACATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_4	CTACACGACGCTCTTCCGATCTNNNNNNNNTTCCAGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_5	CTACACGACGCTCTTCCGATCTNNNNNNNNTTCCTCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_6	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGACATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_7	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGCACTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_8	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGCTGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_9	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGGGATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_10	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGGGTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_11	CTACACGACGCTCTTCCGATCTNNNNNNNNTTGGTGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_12	CTACACGACGCTCTTCCGATCTNNNNNNNNTTTACCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_13	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGTAGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_14	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGTTTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_15	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTAACTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_16	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTACTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_17	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTATCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_18	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTGACTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_19	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTGATTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_20	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTTCCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_21	CTACACGACGCTCTTCCGATCTNNNNNNNNTGTTGATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_22	CTACACGACGCTCTTCCGATCTNNNNNNNNTTAACGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_23	CTACACGACGCTCTTCCGATCTNNNNNNNNTTACACTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_24	CTACACGACGCTCTTCCGATCTNNNNNNNNTTACCTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_25	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCAGTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_26	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCCTATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_27	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCGTATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_28	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCGTTTTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_29	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCTACTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_30	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCTATTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_31	CTACACGACGCTCTTCCGATCTNNNNNNNNTGCTTCTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_32	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGAAGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_33	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGATATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_34	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGATGTTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_35	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGCAATTAGCATGTTATTGCTAGCGTTTTGGCT
ILL_BC_36	CTACACGACGCTCTTCCGATCTNNNNNNNNTGGCATTTAGCATGTTATTGCTAGCGTTTTGGCT
A2_8mer_S	TTCTGTTATTGCTAGCGTTTTGGCTNNKMTGNNKNNKNNKNNKNNKNTGGGTGGAGGAGGTTCTGGAGGTGGTG
A2_9mer_S	TTCTGTTATTGCTAGCGTTTTGGCTNNKMTGNNKNNKNNKNNKNNKNTGGTGGAGGAGGTTCTGGAGGTGGTG
A2_10mer_S	TTCTGTTATTGCTAGCGTTTTGGCTNNKMTGNNKNNKNNKNNKNNKNTGGGTGGAGGAGGTTCTGGAGGTGGTG
A2_11mer_S	TTCTGTTATTGCTAGCGTTTTGGCTNNKMTGNNKNNKNNKNNKNNKNNKNTGGGTGGAGGAGGTTCTGGAGGTGGTG
A2_V5_AS	GAAGAGGATTAGGAATTGGTTTACCGGATCCAGAGGATGGTTCCCATCTCAATGTCAATG
A2_MYC_AS	ACCACCACCACCAGATCCACCACCACCCAAGTCTTCTTCGGAGATAAGCTTTTGTTC
A2_HA_AS	CGTAGTCTGGAACGTCGTATGGGTAGGATCCAGAGGATGGTTCCCATCTCAATGTCAATG
A2_VSV_AS	GTCTATTCATTTCAATATCAGTATAGGATCCAGAGGATGGTTCCCATCTCAATGTCAATG
A2_Vector	TTTTCAATTAAGATGCAGTTACTTCGCTGTTTTTCAATATTTTCTGTTATTGCTAGCGTTTTGGCT

Table S8. Primers used to generate and deep-sequence pHLA libraries.

Letter codes for nucleotides follow the IUPAC naming system. Related to Figure 1.